

Age and growth in the Australian freshwater mussel, *Westralunio carteri*, with an evaluation of the fluorochrome calcein for validating the assumption of annulus formation

Michael W. Klunzinger^{1,3}, Stephen J. Beatty^{1,4}, David L. Morgan^{1,5}, Alan J. Lymbery^{1,6}, and Wendell R. Haag^{2,7}

¹Murdoch University, School of Veterinary and Life Sciences, Freshwater Fish Group and Fish Health Unit, Perth, Western Australia 6150 Australia

²US Department of Agriculture Forest Service, Center for Bottomland Hardwoods Research, Oxford, Mississippi 38655 USA

Abstract: Growth and longevity of freshwater mussels (Unionida) are important for defining life-history strategies and assessing vulnerability to human impacts. We used mark–recapture and analysis of shell rings to investigate age and growth of the hyriid, *Westralunio carteri*, at 5 sites in southwestern Australia. We tested the utility of the in situ marker calcein for validating the assumption of annulus formation in adults. Calcein was incorporated into the shells of all recovered individuals, but it provided an interpretable reference mark in only 4 of 16 individuals. These 4 individuals produced 1 shell ring subsequent to the mark, supporting the assumption of annulus production during the austral winter. Maximum age ranged among populations from 36 to 52 y and maximum size ranged from 72.9 to 82.8 mm. Mean age and length did not differ between sexes, and growth trajectories differed between sexes at only 1 site. Estimates of growth measured by the von Bertalanffy growth constant, K , ranged from 0.021 to 0.336 among sites. Estimates from mark–recapture experiments were 20 to 52% lower than values from shell annuli at all sites except 1 where K from shell annuli was $\sim\frac{1}{2}$ that estimated from mark–recapture. Both methods showed a positive relationship between K and mean water temperature among sites, suggesting a role of riparian shading in regulating stream temperature, and hence, indirectly influencing mussel growth. Mussel growth and mean N or P concentrations were not related among sites, but total N at the site with highest mussel growth was $>2\times$ higher than at any other site. *Westralunio carteri* is a long-lived, slow-growing bivalve. Maximum age, K , and probable age at maturity (4–6 y) are similar to other slow-growing freshwater bivalve groups. This suite of life-history traits is considered an adaptation for stable aquatic habitats. Therefore, *W. carteri* can be expected to adapt poorly to human impacts, such as riparian clearing and water extraction, which increase the temporal variability of environmental conditions in streams.

Key words: life history, growth rate, von Bertalanffy, Hyriidae, Unionoidea, Unionida, Australia

Freshwater mussels (Unionida) are important in freshwater ecosystems because of their filter-feeding, roles in nutrient cycling and biodeposition, and provision of structural habitat for other organisms, and as a food source for a wide range of organisms (Vaughn and Hakenkamp 2001, Spooner and Vaughn 2008). They are highly imperiled throughout the world (Strayer and Dudgeon 2010), and conservation efforts are often compromised by a poor understanding of basic biological variables (Haag 2012). Growth of freshwater mussels varies widely among species and populations, and growth rate affects many other life-history

and population traits, including life span, age at maturity, fecundity, survival, and generation time (Hastie et al. 2000, Haag 2012). Therefore, growth data are essential in ecological studies and for assessing the vulnerability of mussel populations to habitat degradation, harvesting, and other human impacts.

Freshwater mussels and other bivalves generally have prominent, concentric rings in their shells. The rings are analogous to annual growth rings in trees, fish otoliths or scales, and vertebrate teeth. In temperate latitudes, mussels deposit growth rings in response to a reduction or cessa-

E-mail addresses: ³m.klunzinger@murdoch.edu.au; ⁴s.beatty@murdoch.edu.au; ⁵d.morgan@murdoch.edu.au; ⁶a.lymbery@murdoch.edu.au; ⁷whaag@fs.fed.us

tion of growth in winter (Day 1984, Lutz and Clark 1984). The annual formation of these rings has been validated in many studies, particularly in North America and northern Europe, with a variety of methods (reviewed by Haag 2009; see also Dettman et al. 1999, Goewert et al. 2007, Black et al. 2010, Haag and Rypel 2011), and interpretation of shell annuli forms the basis of age and growth information for mussels.

Despite the wide use of shell annuli to estimate age and growth, several uncertainties relating to this technique remain. Little is known about production of annuli in bivalves from regions with less annual variation in temperature than North America and northern Europe, where most growth studies have been conducted. Walker et al. (2001) suggested that freshwater mussels from tropical regions may produce growth checks (i.e., false annuli) in response to maximum summer temperature, aestivation, or seasonal variation in rainfall. Determination of the periodicity of shell ring production typically is accomplished by marking shells, returning animals to the wild, and retrieving them after ≥ 1 y to examine the number of rings produced in the interim. Reference marks often are made by notching or affixing tags to the shell margin, or by using natural disturbance rings produced by handling. These reference marks work well in rapidly growing individuals, but they are difficult to interpret unambiguously for older, slow-growing individuals with tightly crowded annuli (Neves and Moyer 1988, Haag and Commens-Carson 2008). More precise methods involving incorporation of chemical markers into the shell have been used to evaluate annuli production across a wider size and age range of marine bivalves (Kaehler and McQuaid 1999, Fujikura et al. 2003), but these techniques have not been applied widely in freshwater mussels.

Age and growth information is becoming more widely available for the freshwater mussel families Margaritiferidae and Unionidae. This information is central to emerging concepts of life-history evolution and is important in developing effective conservation strategies for these imperiled groups (Bauer and Wächtler 2001, Haag and Rypel 2011, Haag 2012). In contrast, few age and growth data are available for the Hyriidae, which is the dominant freshwater bivalve family in Australasia and much of South America. Most available information is based on untested assumptions of annulus formation, and mussel growth is likely to be influenced by climatic phenomena, such as predictable dry seasons and monsoonal floods (Walker et al. 2001, 2013). Furthermore, many Australian hyriids are of conservation concern because of their restricted distribution and increasing human impacts, such as water withdrawal and salinization (Walker et al. 2001, 2013). Validated age and growth data are needed for this group to provide a broader understanding of life-history diversity in freshwater mussels.

Westralunio carteri is endemic to a small area of extreme southwestern Australia and is the only freshwater mussel known in this region (McMichael and Hiscock 1958, Morgan et al. 2011, Walker et al. 2013). The species has disappeared from much of its former range, but it remains relatively common in suitable habitats. Female *W. carteri* brood larvae from about August to December during the austral spring, but most other aspects of its life history are unknown. Southwestern Australia has a Mediterranean climate with hot, dry summers and cool, wet winters. Winter water temperatures rarely are $<10^{\circ}\text{C}$, making year-round mussel growth a possibility. Extreme summer drought also may influence growth. We used the in situ calcein marker to examine the timing of growth ring deposition in *W. carteri*, and then estimated life span and other growth variables across a range of sites from a combination of mark–recapture and examination of validated annuli.

METHODS

Study sites

We studied age and growth of *W. carteri* at 5 sites in 4 perennial streams in southwestern Australia (Table 1; Fig. 1). The sites varied considerably in physical and water-quality characteristics. Bennett Brook is an unregulated, spring-fed stream flowing through a rural and suburban area and a conservation reserve. It is generally well shaded by an intact riparian overstory and understory, and it has dense beds of emergent aquatic macrophytes that completely cover the stream bed in places. The Brunswick River flows through rural residential, agricultural, and conservation estates and is generally well shaded by an intact riparian overstory and understory. It is regulated by a dam upstream of the study site but also receives considerable groundwater discharge. The Collie River flows through state forest and agricultural land. The amount of riparian shading is variable, but our study site was relatively open. The Collie River is regulated by a major dam that provides perennial flow for irrigation. The Serpentine River is regulated by 2 large dams, downstream of which the river is allocated minimum environmental flows. During our study, these flows were insufficient to maintain connectivity between pools, and much of the river was dry. We had 2 study sites on the Serpentine River. The Horse Drink site was in a privately owned conservation area and was well shaded by an intact riparian zone. The Dog Hill site was largely unshaded and is downstream of the mouth of Birrega Drain, a straightened channel with a history of pollution from industry and agricultural nutrients. In addition to flow regulation and riparian shading, the major differences among these sites are in their annual temperature profiles and the degree of nutrient enrichment (Table 1).

Table 1. Water-quality variables (1974–2009) for streams sampled to assess age and growth of *Westralunio carteri*. Numbers in parentheses following mean values are the number of observations for each variable. Data are long-term monthly mean values from the Western Australia Department of Water gauging stations, except * from MWK, AJL, SJB and DLM (unpublished data). DO = dissolved O₂, TN = total N, TP = total P.

Site	Corresponding gauging station (lat, long)	Salinity (g/L)			Temperature (°C)			TP (mg/L)			TN (mg/L)			DO (%)		
		Mean	Range		Mean	Range		Mean	Range		Mean	Range		Mean	Range	
Bennett Brook	616084 (31°52'39"S, 115°57'34"E)	0.44 (50)	0.10–1.20	18.4 (62)	11.4–24.4	0.12 (243)	0.01–1.20	0.47 (238)	0.47–6.30	61.1 (18)	41.6–77.0					
Brunswick River	612022 (33°13'10"S, 115°55'18"E)	0.21 (108)	0.00–0.70	14.6 (3751)	8.5–25.5	0.49 (79)	0.40–2.10	0.81 (53)	0.50–3.50	82.4 (103)	32.9–134.2					
Collie River	612043 (33°17'57"S, 115°47'58"E)	1.10 (483)	0.39–3.16	17.5 (115,800)	9.1–28.5	0.15 (519)	0.01–1.83	0.56 (494)	0.14–2.93	81.7 (456)	0.0–259.4					
Serpentine River																
Horse Drink	614114 (32°20'13"S, 115°53'06"E)	0.29 (52)	0.19–0.41	16.7 (3950)	11.7–24.8	0.06 (8)	0.03–0.13	0.85 (8)	0.23–2.00	67.9* (24)	44.4–85.6*					
Dog Hill	614030 (32°20'30"S, 115°51'41"E)	0.43 (193)	0.10–2.89	19.1 (464)	9.0–35.2	0.27 (2804)	0.01–2.40	1.73 (2609)	0.15–16.0	96.0 (186)	30.0–174.5					

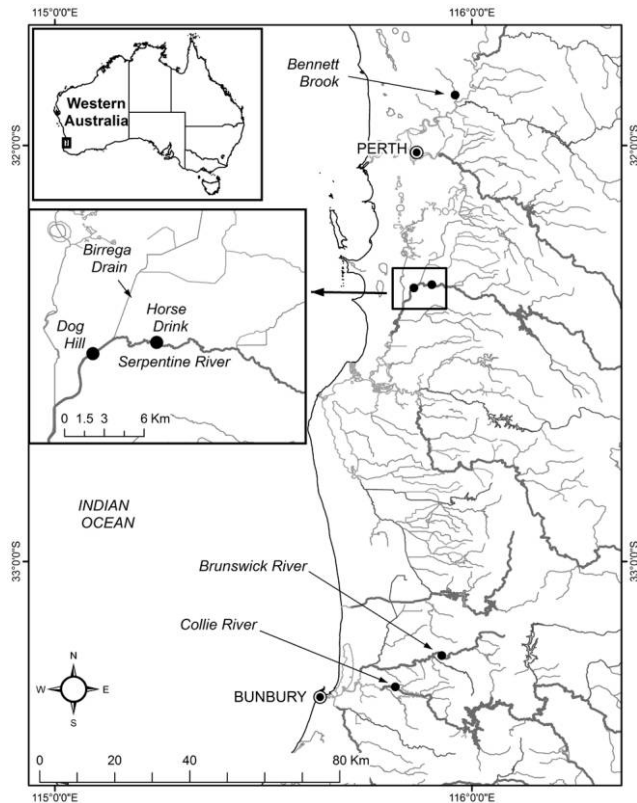


Figure 1. Study sites for estimating growth of *Westralunio carteri*. Data provided by the Western Australia Department of Water.

Validation of annulus formation We used the fluorochrome growth marker calcein to examine the periodicity of growth ring formation during a mark–recapture experiment at the Collie River site. We collected mussels on 24 December 2009 ($n = 16$) and 11 March 2010 ($n = 34$), transported them alive to the laboratory, and affixed an individually numbered plastic shellfish tag (Hallprint Ltd., Hindmarsh Valley, Australia) to each mussel with cyanoacrylate glue. We then immersed mussels in a 250 mg/L calcein solution (SE-MARK[®], Western Chemical, Ferndale, Washington) for 24 h at 5°C, in accordance with the manufacturer’s recommendations. This concentration results in incorporation of calcein in the growing margin of the shell and produces an internal mark visible under ultraviolet or blue light (Kaehler and McQuaid 1999, Eads and Layzer 2002). We held mussels in the laboratory for a maximum of 1 d for marking with calcein and returned them to the stream. We returned to the sites ~1 y later (17 February 2011) and recaptured as many marked mussels as possible. Recaptured mussels were transported to the laboratory, anaesthetized in ice slurry, and tissues were dissected from the shells. To prevent breakage during sectioning, we dried shells briefly and coated them with FR 251 epoxy resin (Fiberglass Resin and Sales, Welshpool,

Australia). We cut and prepared 0.8-mm-thick radial thin sections from each specimen with a low-speed saw with a diamond wafering blade (Buehler Ltd., Lake Bluff, Illinois) following Haag and Commens-Carson (2008) and viewed thin sections under a compound microscope with an interference blue filter for 495/520 excitation/emission wavelengths, which cause calcein to fluoresce. Our assumption of annulus formation was deemed validated if shells had a single growth ring beyond the fluorescent mark corresponding to time since marking.

Mark–recapture estimation of growth and analysis of shell thin sections

We collected *W. carteri* by hand at all sites in February 2010. Sample sizes at each site ranged from 35 to 204 individuals, and we attempted to obtain as wide a size range as possible from each population. To reduce stress, we held mussels at the site in buckets of river water in a shaded location during processing. We affixed an individually numbered plastic shellfish tag (Hallprint Ltd.) to each mussel with cyanoacrylate glue, measured initial total length (nearest 0.1 mm) of each individual, and returned mussels to the substrate. Total processing time was ~8.5 h. We left mussels in the streams for ~1 y, and retrieved as many individuals as possible in February 2011. We transported recaptured mussels to the laboratory, measured final total length of all individuals, and then anaesthetized and dissected them. We determined the sex of each individual by examining the gills. In females, the middle 2/3 of the inner gills are modified as marsupia for brooding larvae.

We prepared thin sections of all recaptured specimens as described previously and examined them under a compound microscope. Sections were observed in random order and blindly with only the tag number and no information about site of origin. We estimated the age of each individual by counting the number of annuli in each thin section. Annuli were distinguished from non-annual rings and other shell features (e.g., disturbance rings) with the criteria given by Neves and Moyer (1988) and Haag and Commens-Carson (2008) and based on the appearance of annuli in the validation component of our study. Briefly, annuli were dark lines that could be traced throughout the shell from the outer margin to the umbo. Disturbance rings were similar in appearance but were not continuous throughout the shell and usually ended a few millimeters from the outer margin (Fig. 2).

Juvenile individuals are necessary for accurate estimates of mussel growth parameters (Haag 2009). Juveniles were rare at all sites and the estimated minimum age of marked individuals ranged among sites from 6 to 9 y. Furthermore, because of shell erosion at the umbo, early growth rings were absent on most adults, which precluded back-measurement of length-at-age for the first 1 to 5 y (see below). To provide more accurate estimates of growth, we

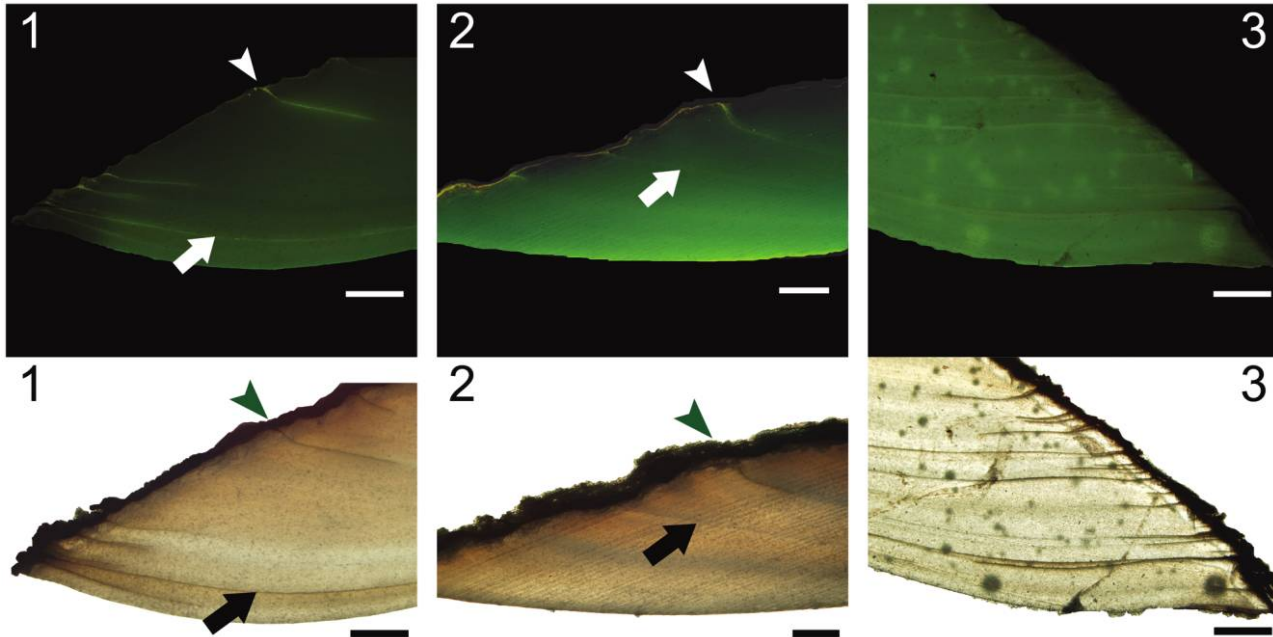


Figure 2. Shell thin sections from *Westralunio carteri* immersed in calcein, returned to the stream, and recaptured 1 y later. Panels with the same number show the same individual photographed under blue excitation (top row) and ambient light (bottom row). Vector arrows indicate calcein marks and block arrows indicate winter annulus. Calcein uptake was not apparent in individual 3 exposed to calcein on the right. Scale bars: 500 μm .

augmented our thin-section samples with uneroded juveniles obtained at various times during the study. We obtained ≥ 1 juvenile (ages 1–4 y; see Discussion) from all sites except the Brunswick River (see Table 2). We back-measured length-at-age for all visible annuli on these specimens following Michaelson and Neves (1995) and Haag and Rypel (2011).

Data analysis We estimated annual growth of individuals in the mark–recapture study at each site in 2 ways. First, we calculated von Bertalanffy growth parameters from Ford–Walford plots (Ricker 1975), which were constructed by regressing final (2011) length against initial (2010) length. The von Bertalanffy parameters α and β were the y -intercept and slope, respectively, of the linear regression from Ford–Walford plots. We estimated the theoretical maximum length, L_{∞} , and the growth constant, K (the rate at which L_{∞} is attained), from the equations $L_{\infty} = (\alpha/[1 - \beta])$ and $K = -\ln\beta$ (Ricker 1975). Second, we estimated growth parameters from shell annuli by constructing von Bertalanffy growth equations from observed age-at-length data for each population.

We compared mean age and mean length between sexes for each locality with 1-way analysis of variance (ANOVA). In the mark–recapture experiment, we used analysis of covariance to examine the effect of initial length

and sex (and the interaction of these factors) on final length. Estimates of K were related to differences in water temperature and nutrient levels among sites by linear regression with all variables $\log(x)$ -transformed. All statistical analyses were completed with SAS (version 9.3; SAS Institute, Cary, North Carolina). We set $\alpha = 0.05$.

RESULTS

Validation of annulus formation

We recovered 16 calcein-marked individuals from the Collie River, all of which appeared to have taken up calcein in the shell and produced a green fluorescent glow under blue excitation microscopy. However, in most individuals, the calcein mark was weak and diffuse and did not indicate a specific location in the shell. In 4 individuals, the calcein marker was distinct and fluoresced vibrantly. These individuals also produced a conspicuous disturbance mark at the same location, and this mark was visible under ambient light. A single growth annulus was present beyond the calcein marker in all of these individuals (Fig. 2). On all 4 validated individuals, the fluorescent mark and associated disturbance ring were located roughly midway between the 2 adjacent annuli, suggesting that considerable growth occurred both before and after marking and that the growing season encompassed much of the austral spring, summer, and autumn (~October–April).

Table 2. Age and growth parameters for 5 populations of *Westralunio carteri*, as determined from annual shell growth in a mark–recapture experiment (Ford–Walford plots) and by estimating age-at-length from shell thin sections. The von Bertalanffy parameters α and β were determined as the y -intercept and slope, respectively, of the linear regression from Ford–Walford plots. Theoretical maximum length, L_{∞} , and the growth constant, K (the rate at which L_{∞} is attained) were determined from the equations: $L_{\infty} = (\alpha/[1 - \beta])$ and $K = -\ln\beta$ (Haag 2009 and Ricker 1975; respectively). L_{\max} = maximum shell length, A_{\max} = maximum age.

Site	No. recovered (No. marked)	No. juveniles	Observed L_{\max}	Observed A_{\max}	Mark– recapture		Shell annuli	
					L_{∞}	K	L_{∞}	K
Bennett Brook	42 (66)	1	72.9	42	66.6	0.080	67.9	0.124
Brunswick River	77 (85)	0	77.0	51	76.4	0.021	75.0	0.044
Collie River	92 (204)	14	73.7	50	62.2	0.139	61.4	0.175
Serpentine River								
Horse Drink	42 (65)	11	78.6	52	66.0	0.090	65.3	0.155
Dog Hill	15 (35)	1	82.8	36	77.0	0.336	80.7	0.170

Age and growth Maximum age ranged among populations from 36 to 52 y, with the shortest life spans recorded from the Serpentine River at Dog Hill and Bennett Brook (Table 2). Maximum size varied little among most populations (72.9–78.6 mm) but was slightly larger at Dog Hill (82.8 mm). Mean age and length did not differ between sexes at any site (age: $F = 0.07$ – 0.99 , $p = 0.33$ – 0.80 ; length: $F = 0.00$ – 1.21 , $p = 0.28$ – 0.97).

Recapture rates of marked individuals varied among sites from 43 to 91% (Table 2). Final length was strongly related to initial length in all populations ($R^2 = 0.965$ – 0.996 , $p < 0.0001$ for all comparisons). Apparent negative growth was observed for a few individuals at all sites except the Serpentine River at Dog Hill. All cases of apparent negative growth occurred in old, slowly growing individuals, and the magnitude of decline in size was never >0.95 mm and averaged 0.17 mm. Final length as a function of initial length did not differ between sexes, and no significant sex \times initial length interaction was observed at any site (sex: $F = 0.97$ – 2.71 , $p = 0.11$ – 0.36 ; sex \times initial length: $F = 0.70$ – 2.17 , $p = 0.15$ – 0.42) except at the Brunswick River where sex and the sex \times initial length terms were significant (sex: $F = 4.53$, $p = 0.04$; sex \times initial length, $F = 4.74$, $p = 0.03$). However, the effects of sex and the interaction term at Brunswick were very small (sums of squares ≤ 1 for both sex and sex \times initial length compared with 2399 for initial length), and predicted final length was essentially identical for both sexes throughout the range of initial lengths.

Growth in the mark–recapture experiment differed widely among populations and estimates of K were highest for the Serpentine River at Dog Hill and the Collie River. Growth estimated from shell annuli showed similar variability with the highest values of K at Dog Hill and the Collie River. However, values of K from mark–recapture

experiments were 20 to 52% lower than values from shell annuli for all sites except Dog Hill where K from shell annuli was $\sim 1/2$ that estimated from mark–recapture. In general, values of K and age-at-length estimates from shell annuli showed that growth of *W. carteri* slowed dramatically after about age 10 and maximum length (L_{∞}) was approached slowly (Fig. 3A–E).

Estimates of K from mark–recapture and shell annuli both were positively related to mean water temperature, but the significance of these relationships was marginal ($p = 0.040$ and $p = 0.077$, for mark–recapture and shell annuli, respectively). However, mean water temperature explained a high percentage of the variation in K among sites for both estimates (mark–recapture: $R^2 = 80.3\%$; annuli: $R^2 = 70.2\%$). Variation in K among populations was not related to mean P or N concentrations for either data set (mark–recapture P: $p = 0.63$, N: $p = 0.49$; annuli P: $p = 0.87$, N: $p = 0.22$, respectively).

DISCUSSION

Westralunio carteri is a long-lived, slow-growing bivalve. Maximum age and values of K are similar to other slow-growing freshwater bivalve groups, such as the family Margaritiferidae and the tribes Amblemini, Pleurobemini, and Quadrulini in the family Unionidae. Median values of lifespan and K for all these groups range from 25 to 50 and 0.10 to 0.17, respectively (Haag and Rypel 2011). *Westralunio carteri* is similar to these groups in having no sexual dimorphism and little difference in growth between sexes, although the small sex-specific differences in growth seen in the Brunswick River could indicate the presence of subtle, quantitative differences in growth trajectories between males and females (e.g., Kotrla and James 1987). Another important similarity to these long-lived groups is the prob-

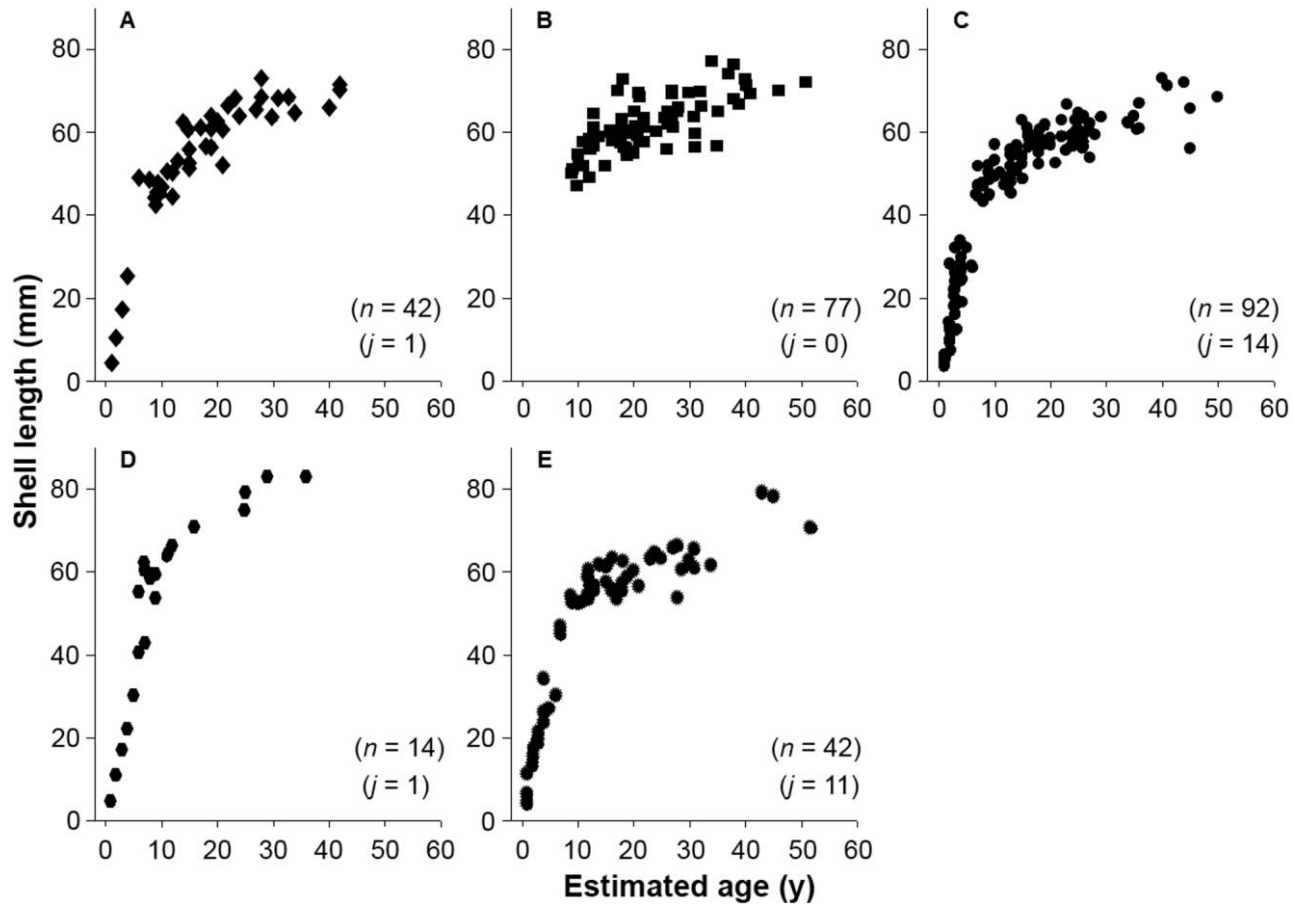


Figure 3. Age-at-length estimates inferred from shell annuli for *Westralunio carteri* in Bennett Brook (A), Brunswick River (B), Collie River (C), Serpentine River, Dog Hill (D), and Serpentine River, Horse Drink (E). The number of adults (n) and juveniles (<4 y old) represented in the data are given in brackets. For information on glochidial size and growth, see Klunzinger et al. (2012, 2013).

able late age at maturity. The average among-population value of K from thin sections in our study (0.134) predicts an age at maturity of ~ 6 y (Haag 2012), and field observations indicate first maturity at ~ 27 mm (MWK, unpublished data), which corresponds to 4 to 5 y of age.

Our validation experiment suggested that *W. carteri* deposited annuli sometime between the austral late autumn and winter (roughly May–August). Calcein was not a consistently effective marker even though we used a staining concentration and duration that produced consistent results in another study with freshwater mussels (Eads and Layzer 2002). Freshwater and marine investigators who achieved high marking success with calcein used rapidly growing larval or juvenile individuals (Eads and Layzer 2002, Moran and Marko 2005). In another study, the percentage of successfully marked marine bivalves declined with increasing age (van der Geest et al. 2011). The low growth rates of adult *W. carteri* could have resulted in poor incorporation of the calcein marker in the shell. Adult *Perna perna*, a marine bivalve, were successfully marked

with calcein (Kaehler and McQuaid 1999), but this species grows much more rapidly than *W. carteri*. Moreover, injection of calcein gave superior results to immersion, which was the method we used. Improved incorporation of a chemical reference mark in adult freshwater mussels may require injection of calcein or use of an alternate marker, such as Sr (Fujikura et al. 2003). Nevertheless, when it did produce a distinct mark, calcein provided greater resolution of the location of the reference mark in slow-growing individuals than has been found for other marking methods, such as shell notching (see Haag and Commens-Carson 2008).

Estimates of K from the mark–recapture experiment were consistently lower than estimates from interpretation of thin sections at most sites. We might have failed to identify some annuli on thin sections, which would result in an overestimate of growth rates. However, it is more likely that estimates from mark–recapture were influenced by 2 sources of bias. First, handling mussels even once can result in small, but statistically significant

reductions in growth (Haag and Commens-Carson 2008). Disruption of growth by handling could have been a cause of apparent negative growth in some individuals, but this phenomenon is probably explained mostly by measurement error of slowly growing individuals. Other authors have reported error in successive shell measurements as between 0.1 and ~ 1.0 mm (Downing and Downing 1993, Haag and Commens-Carson 2008), and all of our estimates of negative growth were within this range. Second, and more seriously, the lack of juvenile individuals in our mark–recapture data set probably resulted in a substantial underestimate of K (see Haag 2009). This source of bias may also have caused the exceptionally low value of K obtained from thin sections at the Brunswick River site because this data set also had no information about juvenile growth.

Despite the high likelihood of bias in growth estimates from mark–recapture, the relative pattern of variation in K among sites was generally similar between methods. This result suggests that mark–recapture could provide coarse approximations of relative growth rates when the examination of thin sections is not feasible. The greater discrepancy between methods at the Dog Hill site on the Serpentine River, where estimates from mark–recapture were $2\times$ higher than those obtained from thin sections, is more difficult to explain. This site is highly regulated and influenced by agricultural runoff, so it might experience greater annual variation in environmental conditions than other sites, and 2010 may not have been representative of long-term growth patterns reflected in thin-section growth histories.

For both methods, growth, as measured by K , was highest at the 2 unshaded sites (Serpentine River at Dog Hill and Collie River), and was particularly high at Dog Hill, which also is enriched by agricultural runoff. Total N at Dog Hill was $>2\times$ that at all other sites, but we found no overall relationship between nutrient levels and mussel growth. These results should be viewed with caution because the water-quality samples available to us were long-term monthly averages, and may not have reflected differences among sites in spring pulses of N, which may be particularly important in influencing growth. Relationships between water temperature and K also support the role of riparian shading in regulating temperature, and hence, indirectly influencing mussel growth. Similar relationships between riparian shading and mussel growth were shown in North American streams (Morris and Corkum 1999). Measures like primary productivity could provide a better and more direct assessment of the role of shading and agricultural enrichment in regulating mussel growth by influencing food availability. Increased growth subsequent to agricultural enrichment, loss of shading, or other factors could have major effects on population dynamics because increased growth rates in mus-

sels are associated with a decline in life span (Haag and Rypel 2011). Life span was lowest at Dog Hill, but our sample size at this site was small, and life span was similar at all other sites.

Despite among-population differences in life-history traits related to higher growth at some sites, overall, *W. carteri* can be considered an equilibrium life-history strategist based on its slow growth, long life span, and late age at maturity (Haag 2012). This strategy has a number of potential implications for the conservation of the species. The long life span may provide a misleading indication of its conservation status, which was recently downgraded on the IUCN Red List from vulnerable to least concern, based partly on the abundance of the species in suitable habitat (Köhler 2011). However, the age structure of most populations is unknown, and we have no information about whether these populations are stable and recruiting or are composed entirely of older, relict individuals. Anthropogenic activities in southwestern Australia have caused riparian degradation, secondary salinization, increased water temperatures, and more-variable flow regimes, all of which are exacerbated by climate change (Morgan and Gill 2000, Silberstein et al. 2012). Like other equilibrium life-history strategists, *W. carteri* probably depends on stable habitats (see Haag 2012) and can be expected to adapt poorly to human impacts that increase the temporal variability of environmental conditions in streams.

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